LOCO: Characterization of Phytoplankton in Thin Optical Layers

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Award Number: N000140410275
http://thalassa.gso.uri.edu/rines/loco/index.html
http://thalassa.gso.uri.edu/rines

LONG-TERM GOALS

Our long-term goal is to understand the ecology of phytoplankton inhabiting coastal shelves, upwelling areas, fjords and banks. We are especially interested in ways in which species-specific properties, including colony size and shape (diatoms) and motility (dinoflagellates) interact with physical mixing processes to regulate spatio-temporal distribution patterns. We wish to understand these processes in sufficient detail to be able to predict bloom dynamics, size structure, and the impact of species-specific characteristics of the phytoplankton on ocean optics.

OBJECTIVES

Our goals within the LOCO DRI program are (1) to thoroughly characterize the phytoplankton community within thin layers and compare it to that outside of layers, (2) to increase our understanding of the importance of species-specific characteristics of the plankton to both ecology and ocean optics, and (3) to expand our understanding of the role that biological-physical processes play in thin layer dynamics.

APPROACH

Under previous ONR funding (N000149610247, N000140210247), we have demonstrated that interactions between physical processes at multiple time and space scales, and the species-specific properties of diatoms and dinoflagellates (*e.g.* size, shape, behavior etc.) are important factors contributing to phytoplankton distribution, bloom dynamics, particle size structure and optical characteristics in the ocean. In order to continue this work within the LOCO framework, we have (1) adapted our earlier protocols for use in the open waters of Monterey Bay (*i.e.* exposed, coastal locations), and (2) developed methodologies that will allow us to collect new kinds of data, so that we can begin to investigate our 'next generation' of questions. In August/September of 2005, and in July 2006, we employed our refined protocols during the LOCO field experiments in Monterey Bay, California. Our primary effort was carried out in close collaboration with Donaghay, Sullivan, Holliday and Hanson, working from *R/V Shana Rae*. We are fortunate to also have the opportunity to collaborate with the many additional PIs in the LOCO program.

Report Documentation Page

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
2009		**00-00-2009 to 00-00-2009**

4. TITLE AND SUBTITLE	5a. CONTRACT NUMBER
LOCO: Characterization Of Phytoplankton In Thin Optical Layers	5b. GRANT NUMBER
	5c. PROGRAM ELEMENT NUMBER
6. AUTHOR(S)	5d. PROJECT NUMBER
	5e. TASK NUMBER
	5f. WORK UNIT NUMBER
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) **University of Rhode Island,Graduate School of Oceanography,South Ferry Road,Narragansett,RI,02882**	8. PERFORMING ORGANIZATION REPORT NUMBER
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)	10. SPONSOR/MONITOR'S ACRONYM(S)
	11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release; distribution unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT
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15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			
unclassified	**unclassified**	**unclassified**	**Same as Report (SAR)**	**11**	

WORK COMPLETED

Our efforts in FY09 were divided between LOCO activities (further analyses of samples collected during the 2005 and 2006 LOCO field experiments, preparation of manuscripts, etc.) and continuing evaluation and development of the capabilities of our CytoSense scanning, in-line flow cytometer, which can be used both bench-top and *in situ* - http://www.cytobuoy.com/

LOCO Sample Analysis. Our sample set from the LOCO experiments includes over 300 preserved whole water samples collected from inside and outside thin layers and surface slicks, about 30 offshore samples from *R/V New Horizon* (2005), live counts (2005) of the fragile dinoflagellate *Akashiwo sanguinea* (which does not reliably preserve), over 100 filter samples for epifluorescence-based image analysis of picoplankton, over 20 hours of videotaped record of microscopic examination of live phytoplankton, and about 70 CytoSense samples (2006). Analysis of samples has been prioritized to address specific questions, and to interact with other members of the LOCO team. We have completed several manuscripts, and have others in preparation.

CytoSense/CytoSub Evaluation. We continue to develop methodologies for using this exciting instrument to further the long-term goals of our research program. In FY09, our objectives were to:

- Deploy CytoSense *in situ*
- Obtain *in situ* profiling data
- Operate at multiple gain settings, in order to characterize different size classes of particles
- Evaluate our ability to match CytoSense data to species-specific, microscopy-based observations made from discrete water column samples, as well as to *in situ* optical data.
- Evaluate the potential to use CytoSense with fluorochrome dyes, in order to address an entirely new set of questions.

RESULTS

LOCO. Monterey Bay is home to an extraordinarily diverse community of phytoplankton. Although many of the same taxa were present in both 2005 and 2006, the dynamics of each year were quite different, and were correlated to hydrographic patterns. Our extensive data set, coupled with those of our colleagues, allows us to address many of our originally proposed questions, including:

- Are thin layers composed of an enhanced concentration of the total integrated phytoplankton community, or are they dominated by a particular taxon, or size/shape class?
- Are spatially extensive layers taxonomically uniform?
- Are all taxa and size classes of phytoplankton capable of forming layers?
- Are motile phytoplankton more likely to form layers?

Once characterized, the implications of these features of the water column can be explored. Thin layers are of interest for multiple reasons, including their potential impact on both the oceanographic environment, and Navy sensor systems. For example, high-biomass thin layers affect the inherent optical properties (IOPs) of the water column (Sullivan *et al.* 2005, Sullivan *et al.* in press), and because they offer dense concentrations of food potentially attractive to zooplankton and planktivorous fish (acoustical scatterers), they also impact ocean acoustics (Holiday *et al.* 2003, Holliday & Stanton 2005). But we also found that several low-abundance taxa appeared to be restricted to narrow depth

intervals in the water column. Even at low densities, they may constitute functional thin layers, with large ecological impact even if their biomass is too low to dominate an optically defined thin layer. Examples include the toxic HAB taxa *Alexandrium catenella* and *Dinophysis fortii*. Moreover, concentration into thin layers may facilitate obligatory relationships between taxa, such as the hypothesized interrelationships between cryptomonads, *Myrionecta rubra*, and *Dinophysis* spp., all of which were observed in this system. Therefore, from the perspective of the organism, thin layers can be viewed as *critical scale* phenomena, which may be essential to their ecological success.

CytoSense Evaluation. Our CytoSense scanning, in-line flow cytometer was specifically designed to study the size, shape, physiological and optical properties of phytoplankton colonies and individual cells within the colonies, but it can also be used for the more traditional analyses of small, single cells. It operates in both bench-top, and *in situ* mode. It streams near-real-time, multi-channel data on the size and optical properties of each particle as it flows past the sensors, creating a detailed scan of the variations in complexity of each parameter over the length of the particle. Our instrument contains a blue (488nm) laser, and sensors to measure forward scatter, side scatter, red, orange, yellow and green fluorescence, and curvature. We are using this instrument in two ways: (1) to analyze small (*e.g.* < 5 µm), single cells, which tend to be ubiquitous and numerically dominant in marine environments, and (2) in our continuing investigations of the interactions between cell/colony size and morphology of the larger phytoplankton (*e.g.* diatoms and dinoflagellates), small-scale turbulence, and optics. By varying the gain settings on the instrument, we can choose to focus on different size/abundance classes of phytoplankton.

Prior to employing CytoSense as a critical part of our new research program in East Sound, WA, we deployed the instrument in Narragansett Bay, RI for testing. For *in situ* use, the instrument is housed in an underwater casing, and is connected to the shipboard computer via a sea cable (Figure 1). *In situ* data collected in a deployment at a high gain setting (targeting small particles) is shown in Figure 2, and characteristically finds that the numerically dominant particles in the ocean are very small (~1 µm). But research efforts in my lab are primarily focused on the much larger and less abundant diatoms and dinoflagellates, and by setting the instrument to a low gain setting, we can focus on these particles. CytoSense is ideal for studying the long, complex and beautiful colonies of diatoms that are of the greatest interest to us (Figure 3)!

In our May 2009 field experiment in East Sound, WA, CytoSense was extensively used in conjunction with three separate, but closely related field programs. We employed the instrument both on deck, and *in situ* (Figure 4). Examples of *in situ* CytoSense data from East Sound are shown in Figure 5, but the scientific results of this project are primarily reported upon in the Annual Report of Donaghay, Rines & Sullivan. We were thrilled that CytoSense proved so effective at collecting data, that we were able to do far more than allowed for in our original experimental design!

We now turn our attention to the possibilities of using CytoSense in an entirely different way, by incorporating fluorochrome dyes for the study of both intracellular and colonial structures and processes. These compounds can be used with both epifluorescence microscopy, and flow cytometry. One example is SYBR Green, which readily penetrates living cells, and binds with nucleic acids. The DNA-SYBR complex absorbs blue light, and emits green light, and is thus compatible with CytoSense's optical systems. This technique can be used to address questions related to phytoplankton cell cycles and ploidal levels. This data is relevant to elucidating the developmental mechanisms that regulate colony morphology and size, which ultimately impact both particle, and *in situ* optics. Figure

6 depicts SYBR stained material visualized with microscopy, and Figure 7 demonstrates that CytoSense can quantify SYBR fluorescence, and thus will be invaluable in this kind of research.

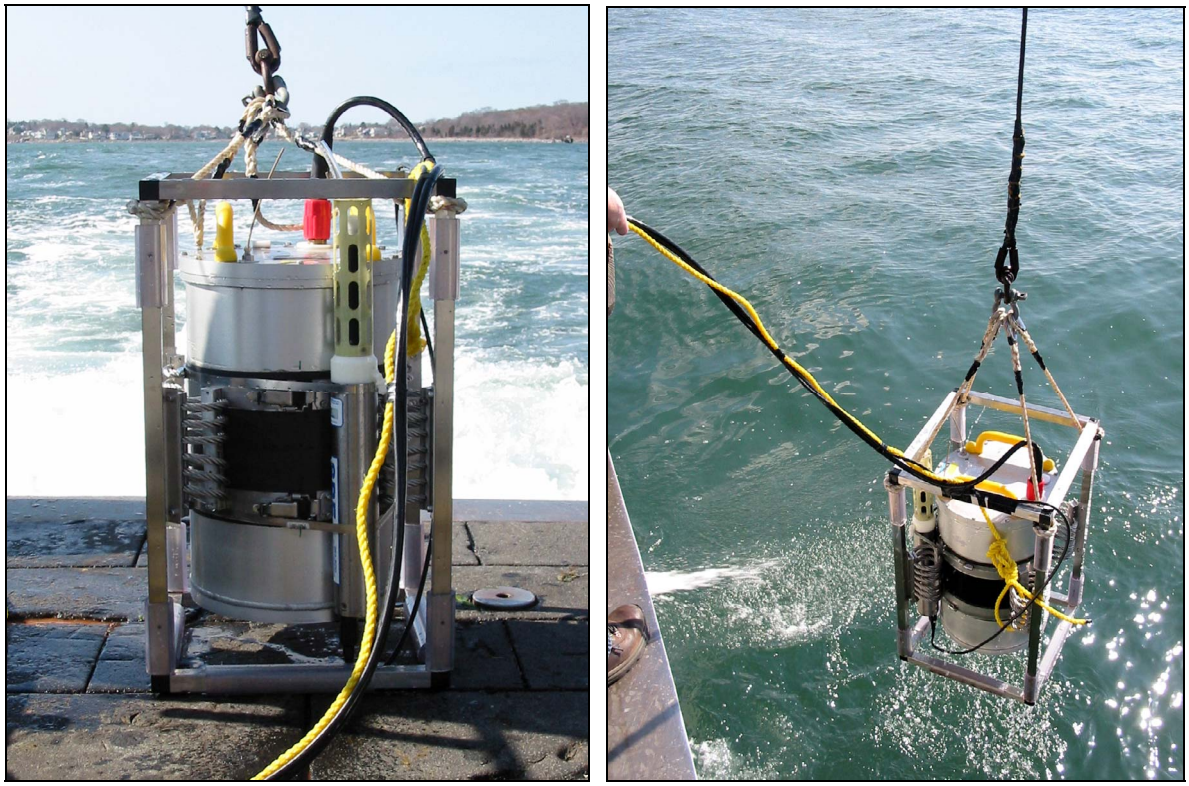

Figure 1. Deployment of CytoSense in Narragansett Bay, Rhode Island, April 7, 2009. Left, the instrument sits on deck in its underwater housing. Right, the instrument is lowered into the water, connected to the on-deck computer via a data cable.

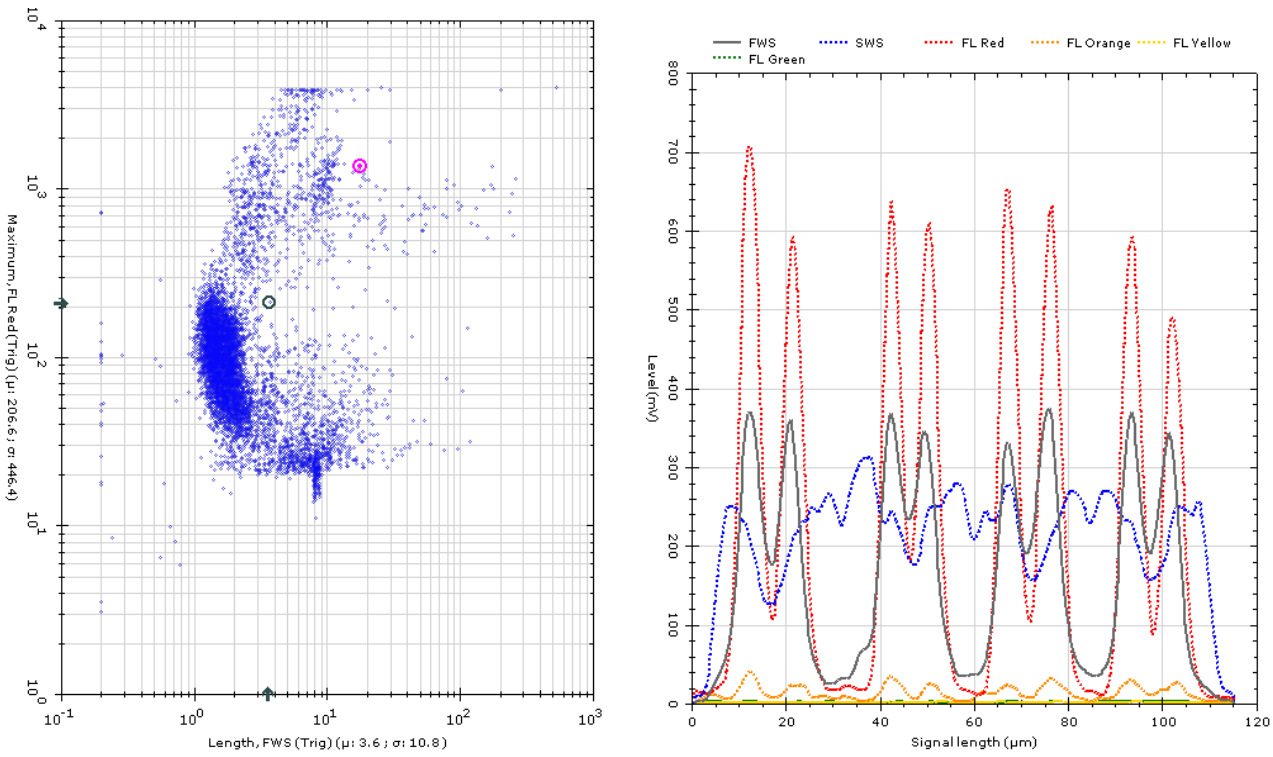

Figure 2. Examples of in situ data collected in Narragansett Bay on April 7, 2009. Left, the graph summarizes the particles quantified and they are plotted as maximum fluorescence in the red channel vs. particle length. There is a large cluster of ~1 µm particles consistent with cyanobacteria such as Synechococcus or Prochlorococcus. Large, chain-forming diatoms are located in the upper right part of the plot, and are numerically far less abundant. Right, an individual pulse profile of one of the chain-forming diatoms. The data depicts a four cell chain, and because of the bi-modal pattern of both forward scatter and chlorophyll fluorescence, it appears that each cell is in the process of dividing. Side scatter persists throughout the pulse, suggesting complex structures.

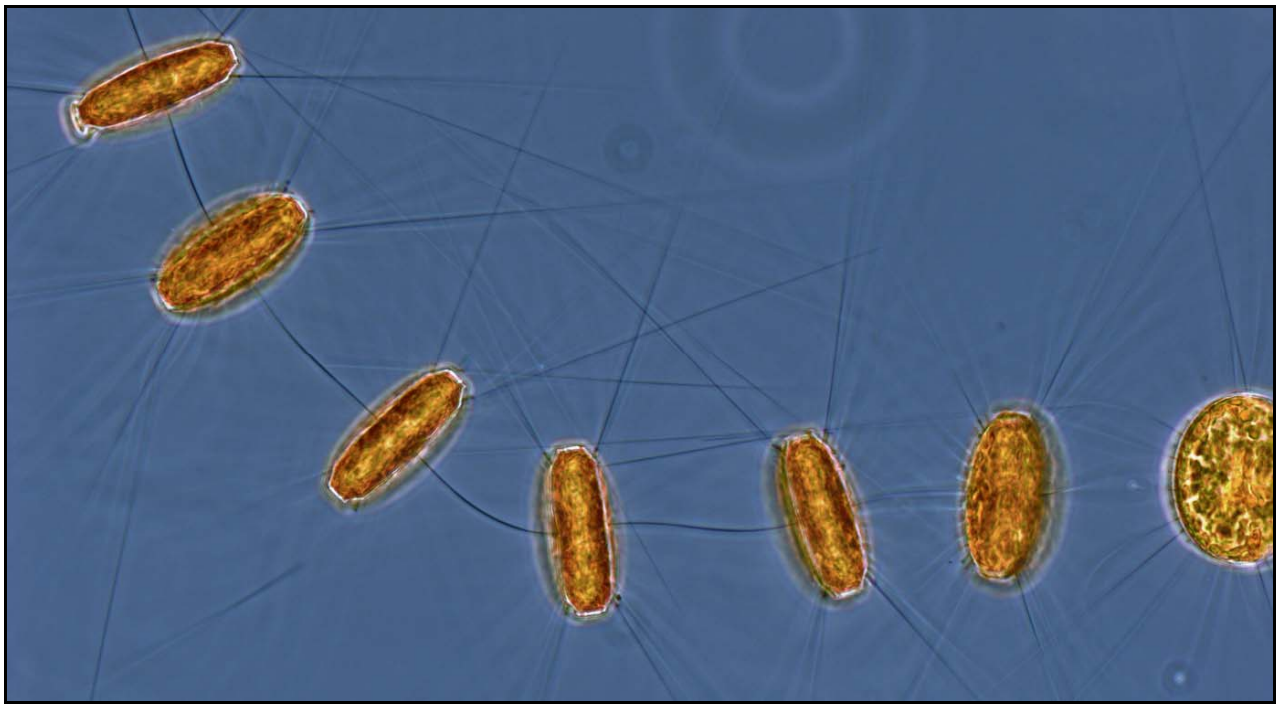

Figure 3. This Thalassiosira is a beautiful example of the large, chain-forming diatoms, which were present in Narragansett Bay during CytoSense testing. The numerous chitan threads emanating from each cell may contribute to the side scatter signal. This taxon, or a similar one is likely to be the source of the pulse profile shown in Figure 2. However, we would be able to make a far more precise match of the data from CytoSense to the organisms in the water by adding CytoBuoy's 'Imaging in Flow' module to our unit.

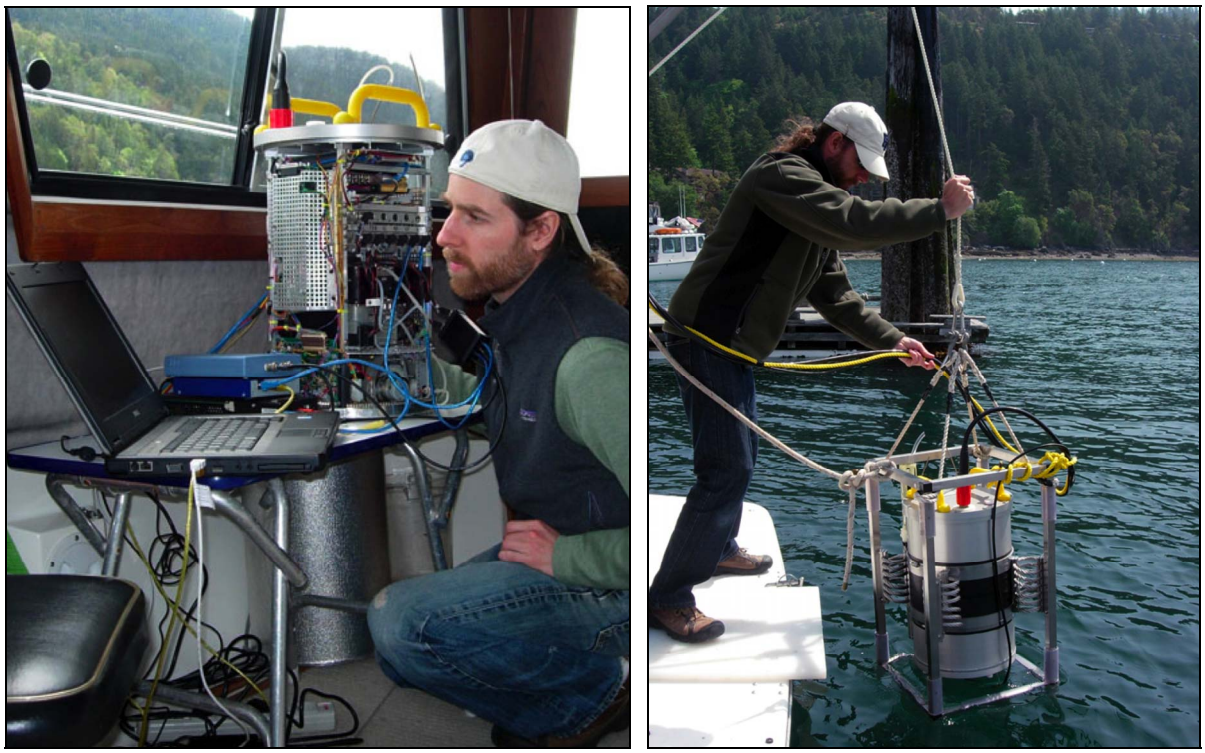

Figure 4. CytoSense proved invaluable to our East Sound experiments. Left, discrete water samples are run through the instrument on deck. Right, CytoSense is deployed from the dock for testing prior to successful experimental deployment in open East Sound waters.

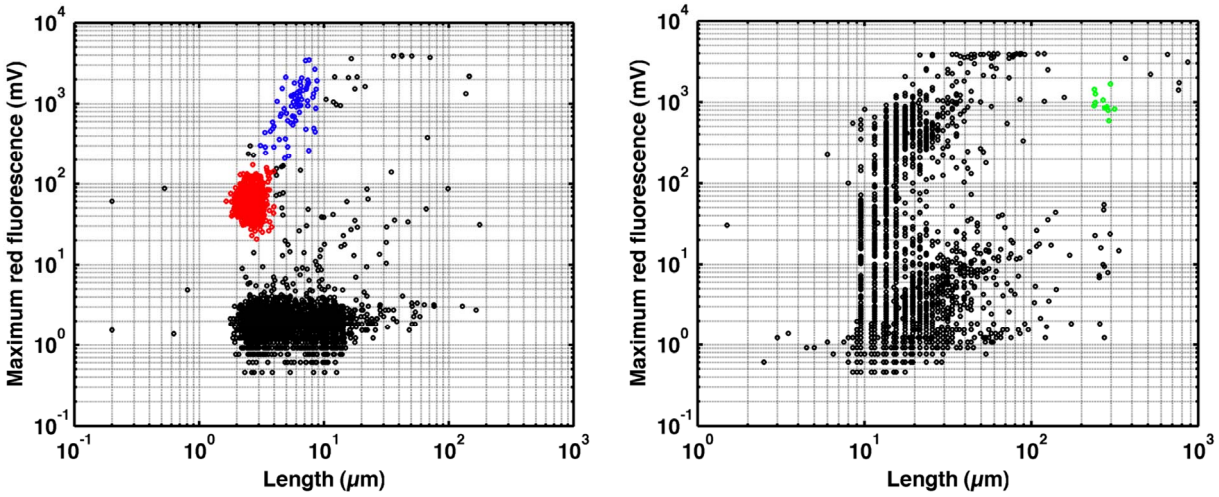

Figure 5.* An example of *CytoSense data from an in situ deployment in East Sound, in this case from 6.7 meters on May 25, 2009. Data was collected at two different gain settings in order to resolve different size classes of particles. The graphs summarize the particles quantified and are plotted as maximum fluorescence in the red channel vs. particle length. Left, cluster highlighted in red depicts ~3 µm cells at 5739 cells/ml and cluster highlighted in blue depicts ~6 µm cells at 442 cells/ml. Right, cluster highlighted in green depicts cells of the diatom Haslea cf. wawrikae.

Many of the morphological and cytological features that can be seen in the microscope (*e.g.* Figures 3 and 6), are also depicted in CytoSense's pulse profiles (Figures 2 and 7), and can be quantified. Thus, CytoSense can be used to collect quantitative data in laboratory and field settings, which will allow us to pursue research on the impact of turbulence on chain forming diatoms, and on the optical properties of individual particles. We will use this information to (1) increase our understanding of the species-specific biology of the organisms, and (2) their impact on the IOPs of the water column.

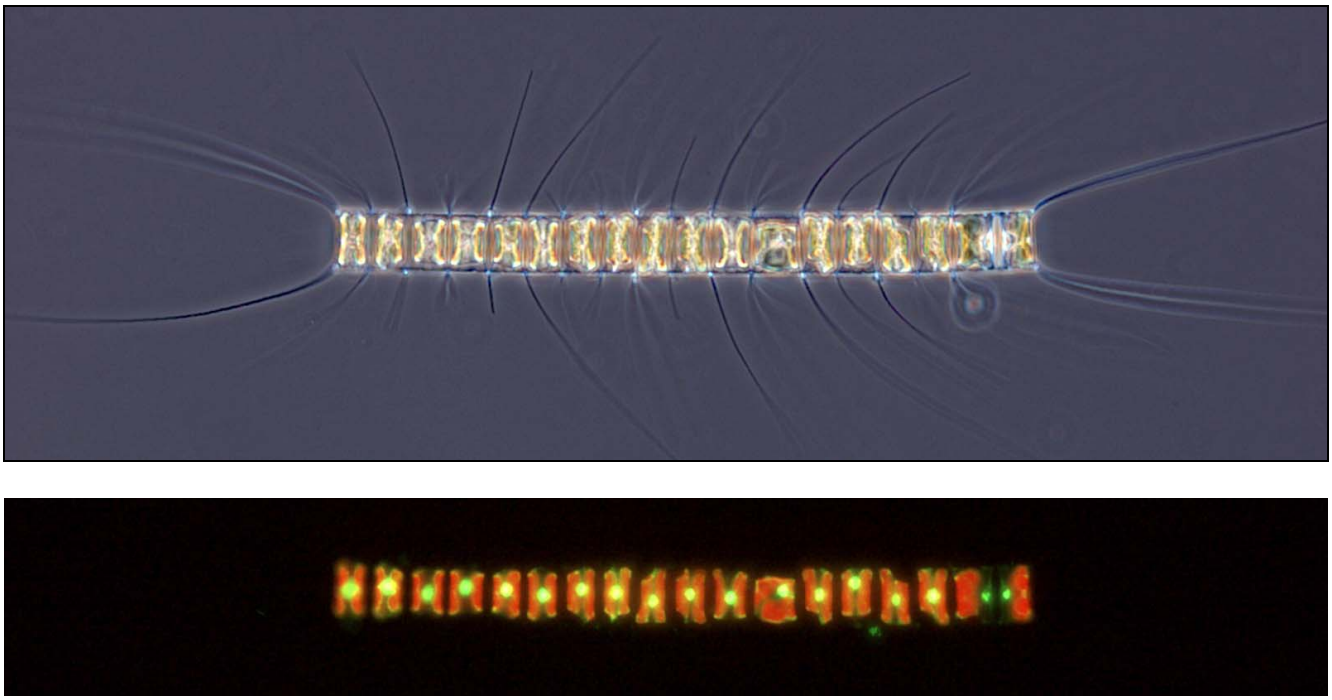

Figure 6. Light micrographs of a colony of Chaetoceros vanheurckii, isolated from East Sound, WA. This material has been stained with the fluorochrome dye SYBR green. Top, colony viewed with phase contrast microscopy. Note the siliceous spines, or setae. The cell at the right end of the chain is in the process of mitotic division. Bottom, the same colony viewed with epifluorescence microscopy. Each cell's two chloroplasts glow red. The SYBR green stained nuclei are yellow/green. Note the recently divided nuclei are smaller than the others in the chain, indicative of their different cell cycle phase.

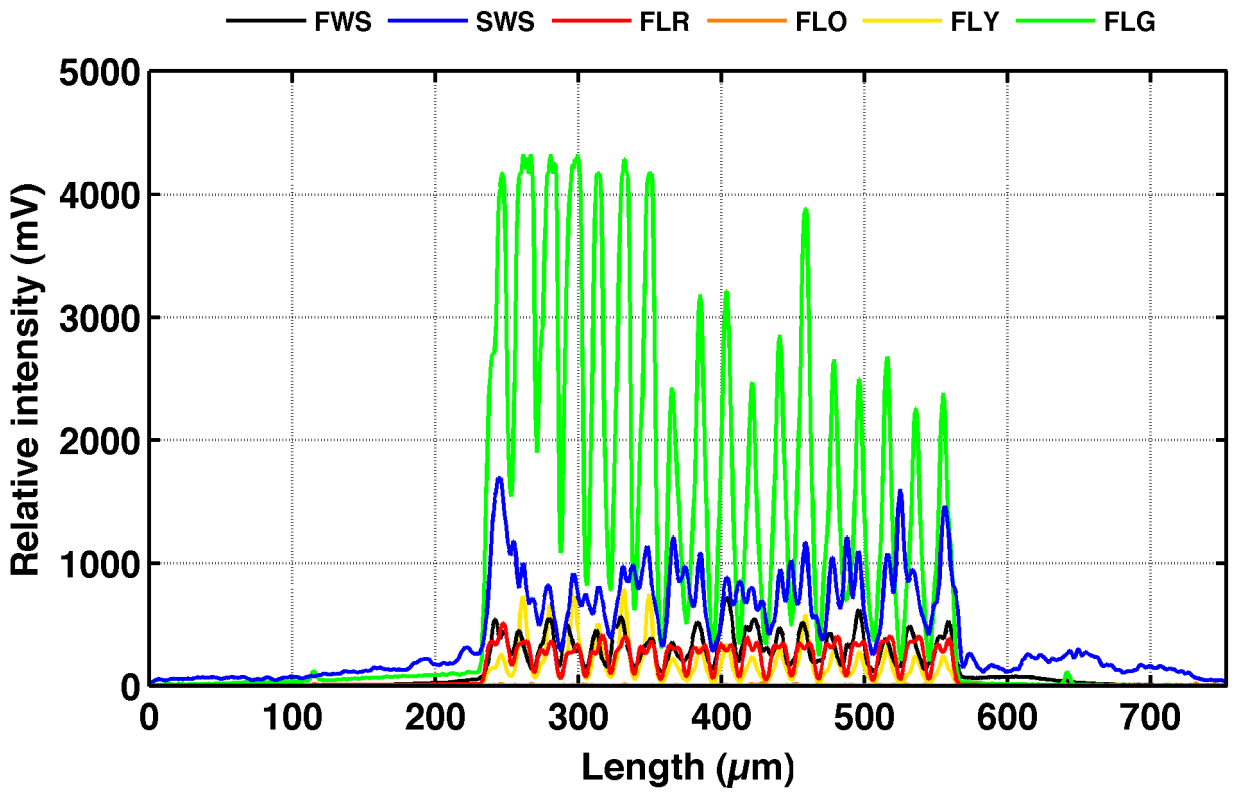

Figure 7. A CytoSense pulse profile of a SYBR Green stained Chaetoceros vanheurkii colony. This is the same sample, but a different colony than the one depicted in Figure 6. The blue trace shows the large amount of side scatter generated by the siliceous setae (spines). Red depicts chlorophyll fluorescence. The very strong green channel signal is the SYBR stained nuclear material. In this example, the left half of the colony has generated almost twice the signal as the right half, suggesting that the cell cycle phases of the two ends of the colony are offset.

APPLICATIONS

Thin Layers of phytoplankton are an important feature of the coastal ocean. However, they don't exist in isolation – they are a component of the biological and hydrographic dynamics of the entire water column, and must be studied as such. Thin layers may simply contain an enhanced concentration of the phytoplankton community found throughout the water column, but frequently they contain a unique flora, with layers at different depths dominated by different taxa. Patterns exist at multiple scales. In addition to species-specific differences, we have demonstrated that *groups* of organisms (*e.g.* diatoms, dinoflagellates and picoplankton) can exhibit separate patterns of vertical distribution, thus different processes must regulate their dynamics. These relationships are not static: layers of motile organisms may migrate in and out of other structures. Thus, there may be many simultaneously occurring and kaleidoscopically interacting patterns, operating on multiple spatio-temporal scales.

Our LOCO data provides a wealth of information on the species-specific distribution of phytoplankton inside and outside of thin layers in Monterey Bay. When collaboratively combined with the physical, chemical, optical and acoustical data of our LOCO colleagues, we have a unique opportunity to further

our knowledge of both the mechanisms of thin layer formation, maintenance and dissipation, and the biological, ecological and optical impacts of those layers.

Species-specific properties of phytoplankton such as size, shape, pigment composition, biomineralization and toxin production are known to play important ecological and oceanographic roles. However, the classical 'form and function' questions remain largely unanswered (Sournia 1982), and to my mind are amongst the most fascinating in biological oceanography. I am especially interested in the interactions between phytoplankton morphology (at both colony, and subcellular levels), physical mixing processes operating at the scale of the organism, and optics. Our CytoSense flow cytometer gives us a new, innovative tool with which to pursue the significance of particle variability with respect to biological/ecological questions, and also from the perspective of impact of species-specific properties of the phytoplankton on ocean optics. This instrument does not replace a microscope – its tremendous power lies in generating data to link IOPs to the highest quality, detailed microscopic images that we can obtain of the organisms themselves. We believe that CytoSense can quantify the optical properties of plankton is such a detailed way that it will both revolutionize our studies of phytoplankton ecology, and provide data critical to linking microscope-based studies of the species-specific properties of phytoplankton to the *in situ* inherent optical properties (IOPs) measured by our team.

RELATED PROJECTS

In the LOCO program, we are working closely with Donaghay & Sullivan to link species-specific patterns of plankton distribution to physical and optical data. We are working with other members of the LOCO team to provide information on the phytoplankton in Monterey Bay to help them interpret their data sets.

The CytoSense development portion of this project is highly relevant to two of our additional ONR projects: *In situ* quantification of the impact of episodic enhanced turbulent events on large phytoplankton (Donaghay, Rines & Sullivan), and *In situ* validation of the source of thin layers detected by NOAA airborne fish lidar (Donaghay, Sullivan, Rines & Churnside. We also collaborated closely with Dr. Alan Weidemann (NRL, Stennis), who was conducting a related project as part of this effort. During May 2009 field work in East Sound, Washington, we extensively utilized our CytoSense flow cytometer to pursue the goals of these projects, both on deck, and *in situ*.

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PUBLICATIONS

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Sullivan. J.M., Donaghay, P.L., **Rines, J.E.B.** Coastal thin layer dynamics: consequences to biology and optics. Continental Shelf Research *Special Issue*: The Ecology and Oceanography of Thin Plankton Layers, accepted, refereed.

McFarland, M., **Rines, J.**, Donaghay, P. Use of automated image analysis to quantify the distribution of photosynthetic picoplankton relative to thin layers in Monterey Bay, CA. Marine Ecology Progress Series, in review, refereed.

Graff, J.R., **J.E.B. Rines** & P.L. Donaghay. Bacterial colonization of phytoplankton in the pelagic marine environment. Marine Ecology Progress Series, in review, refereed.

PRESENTATIONS

Graff, J.R., **J.E.B. Rines** & P. Donaghay. Quantification of bacteria attached to phytoplankton in Monterey Bay, California USA. ASLO, Nice, France, January 2009.